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The ACE (I/D) polymorphism and the RAAS in type 1 diabetes mellitus

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Chapter 6

Blood pressure, BRS and HRV responses to norepinephrine in healthy subjects with contrasting ACE (I/D) -genotype

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Submitted.

Abstract

Aim/hypothesis. Blood pressure regulation is multifactorial, including the RAAS and autonomic nervous system, with important interactions between these systems. The DD-genotype of the ACE (I/D) polymorphism is associated with increased ACE levels and altered vascular reactivity and autonomic function. We studied effects of the ACE polymorphism on the interaction between both systems, by investigating blood pressure and heart rate responses to norepinephrine (NE) in 24 healthy subjects with contrasting ACE genotypes.

Methods. NE was infused at doses of 20, 40, 80 and 160 ng/kg/min, 30 min each. Blood pressure (finapress) and pulse rate (ECG) were measured continuously, baroreflex sensitivity (BRS) was calculated using a transfer function method. Heart rate variability (HRV) analysis was performed by discrete Fourier transformation of the inter-beat intervals. NE caused similar increase in blood pressure in both groups.

Results. Heart rate decreased more pronounced in the DD group. At baseline, BRS was similar in both groups. NE infusion increased BRS in both groups, this increase was larger in the DD group at 20 and 80 ng doses, the overall difference in BRS between the groups was close to significance ($P=0.07$). Total power of HRV increased during the NE infusions in both groups, primarily due to increased HF-power. The LF/HF ratio decreased, indicating increased parasympathetic activity. NE infusions induced larger baroreceptor mediated fall in heart rate in healthy DD subjects, which was further substantiated by BRS measurements.

Conclusions. DD subjects potentate their BRS more effective during a NE-challenge, which might protect them against potential increased adrenergic vascular reactivity.

Introduction

The renin-angiotensin-aldosterone system (RAAS) plays a main role in the regulation of blood pressure. The genetic diversity of the RAAS is becoming increasingly clear, with several genetic polymorphisms for renin, angiotensinogen, ACE, and the AT₁ receptor. The insertion/deletion polymorphism of the gene encoding for ACE is associated with plasma and tissue levels of ACE, with the highest levels in DD subjects (1;2). The elevated ACE levels in DD homozygotes are associated with enhanced responses to exogenous angiotensin (Ang) I in healthy and diabetic subjects, supporting the assumption that the higher ACE-levels have physiological significance (3-5). Accordingly, one might expect a

higher blood pressure in DD homozygotes, but - with the exception of scattered reports - most studies do not reveal a relationship between ACE genotype and blood pressure (6;7).

However, association studies are subject to many drawbacks (8). A different approach would be, to explore whether differences in blood pressure regulating systems are present between subjects with different genotypes. Considering the multifactorial nature of blood pressure regulation, it would be logical to assume that a genetically facilitated generation of AngII would lead to compensatory responses of other neurohumoral systems that offset a tendency towards higher blood pressure. In support of this assumption, *in vitro* studies in human blood vessels revealed differences in the responsiveness to phenylephrine, as well as in the contribution of the NO system in vascular tone between DD and II homozygotes (9). *In vivo*, an enhanced reactivity to phenylephrine in DD genotype was reported during extracorporeal circulation (10). Furthermore, during angiotensin infusion in man, differences in baroreflex-mediated changes in heart rate were found (11), and in twins the DD genotype was associated with a higher heart rate variability (12). Taken together, these findings suggest that the impact of ACE genotype on blood pressure regulation extends beyond the RAAS, and results in differences in vascular reactivity and cardiovascular reflexes between subjects with different ACE genotype.

In the present study, therefore, we investigated the responses of blood pressure and heart rate to norepinephrine (NE) infusions in healthy subjects with contrasting ACE genotypes, and evaluated baroreflex sensitivity (BRS) and HRV during this intervention.

Materials and Methods

Subjects

The study participants were healthy subjects, aged between 18 to 40 years, with no medical history of cardiovascular disease. They had a normal blood pressure (systolic < 140 mmHg and diastolic < 85 mmHg), were non-obese (body mass index (BMI) < 27 kg/m²) and were non-smoking. Twenty-four subjects with contrasting ACE-genotypes (n=12 each, denoted as II group and DD group) were individually matched for sex, age (within 3 years) and BMI (within 2.5 kg/m²). The subject characteristics are shown in table 1. The groups only differed in their serum ACE-level ($p < 0.01$). The study was approved by the local medical ethics committee and all participants gave written informed consent to participate in the study after explanation of the purpose of the study.

Experimental design

The measurements were carried out in a quiet temperature-controlled (22°C) room with the participants in the supine position. Subjects reported at the research unit at 7.30 am, after an overnight fast. Two intravenous catheters were inserted into the antecubital veins. One was used for infusion of norepinephrine (NE) and 100 ml h⁻¹ of saline (0.9%), the contra lateral catheter was used for blood collections. After acclimatization to the study conditions, a baseline measurement of 30 min was performed. Thereafter, NE was infused at 4 incremental doses of 20, 40, 80 and 160 ng kg⁻¹ min⁻¹, with each infusion step lasting 30 min. Blood pressure was recorded every 5 min with a semi-automated device (Dinamap 1846, Critikon, Tampa, FL, USA). Pulse rate was recorded continuously on an electrocardiography oscilloscope (Hewlett Packard, Boblingen, Germany). Blood samples were taken at baseline and at the end of each infusion period. Water was the only liquid allowed to drink during the study period. Plasma samples for determination of NE, were collected in tubes containing 10% EDTA, immediately centrifuged at 4°C and stored at -20°C until HPLC analysis (13). Serum ACE activity was measured as the generation of Hip from the substrate Hip-His-Leu. Normal values in our laboratory range from 10 to 40 U l⁻¹ (14).

Baroreflex sensitivity and heart rate variability measurements.

A Finapres cuff (Finapres, Ohmeda 2300, Englewood, Colo., USA) was applied to the midphalanx of the third finger for continuous blood pressure monitoring. These measurements were performed at baseline and during each infusion step. Baroreflex sensitivity (BRS) was determined by the transfer function technique, using the CARSPAN program, as described earlier (15-17). This program performs discrete Fourier transformation of non-equidistant samples of blood pressure and RR interval series. The time-series were corrected for artifacts and checked for stationarity before the spectral analysis. BRS was defined as the mean modulus between spectral values of systolic blood pressure variability and heart rate variability in the 0.07 to 0.15 Hz frequency band with a coherence of more than 0.3. BRS is expressed in ms mmHg⁻¹. A BRS of 10 ms mmHg⁻¹ indicates that a rise of 1 mmHg in systolic blood pressure will induce 10 ms of RR interval lengthening. The coefficient of variation of this method is 13% (15). At least three BRS-estimations of 100 to 300 s were determined during each infusion step and averaged for statistical analysis.

Heart rate variability (HRV) analysis was done by discrete Fourier transformation of the inter-beat intervals, and was performed according to the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing

and Electrophysiology (18). The total power of HRV was assessed in the 0.02 to 0.40 Hz frequency band. HRV power was divided in a low (0.04 to 0.15 Hz) frequency (LF) power spectrum, and a high (0.15 to 0.4 Hz) frequency (HF) power spectrum. The ratio of the LF and HF power spectra was calculated, as a measure of sympathetic/ vagal balance, i.e. a decrease in LF/HF ratio implies a shift towards parasympathetic activity (19).

Statistical analysis.

Power spectral values have a skewed distribution that is normalized after logarithmic transformation. Therefore, the natural logarithm of BRS and HRV values is used in the analyses. Repeated measures ANOVA was used to compare the blood pressure, heart rate, HRV and BRS responses within and between the *DD* and *II* groups. Student t-test was used for other between groups comparisons. Systolic BP responses were principally analyzed in agreement with the systolic BP based BRS calculations in the spectral analysis methodology. The relationship between the increase in SBP and decrease in heart rate was compared between the groups using the sign test on rank differences (20). A two-sided *p*-value <0.05 was considered significant.

Results

Blood pressure and heart rate

Infusion of NE caused a dose-dependent increase in SBP (table 2, $p < 0.001$) and a decrease in HR (table 2, $p < 0.01$) in both groups. The changes in mean arterial pressure (MAP) were in agreement with those in SBP (table 2). The NE concentrations during all the infusion steps were similar in the *II* and *DD* groups (table 2). The increase in SBP (%change from baseline) was similar among *II* and *DD* subjects (fig.1A). However, the decrease in HR (% change from baseline) was larger in the *DD* subjects compared to *II* subjects (overall difference $p < 0.05$, fig.1B). This resulted in a steeper relationship between change in systolic blood pressure and change in heart rate in the *DD* group compared to the *II* group, as shown in figure 2 ($p < 0.05$ *II* vs *DD*).

Table 1: Clinical characteristics of the study groups.

	II Group	DD Group
Number (n)	12	12
Age (yr)	24 ± 1	25 ± 1
Gender (m:f)	8:4	8:4
BMI (kg/m ²)	24.4 ± 1	22.9 ± 1
Serum ACE (IU/l)	21.4 ± 1	36.8 ± 2*
Systolic pressure (mmHg)	119 ± 3	119 ± 3
Diastolic pressure (mmHg)	68 ± 2	70 ± 2
Mean arterial pressure (mmHg)	85 ± 2	86 ± 2
Heart rate	65 ± 3	65 ± 2
Baseline BRS (msec/mmHg)	14.3 ± 1	14.0 ± 1

Group II and DD: healthy subjects homozygous for the I-allele and D-allele of the ACE-gene polymorphism, see text. Values: Mean ± SEM; * $p < 0.01$ DD vs II.

Figure 1. Percentage change of systolic blood pressure (SBP) and heart rate (HR) during each norepinephrine (NE) infusion dose. Data: mean ± SEM; *P*-values: represent overall change in groups DD versus groups II by repeated measures ANOVA.

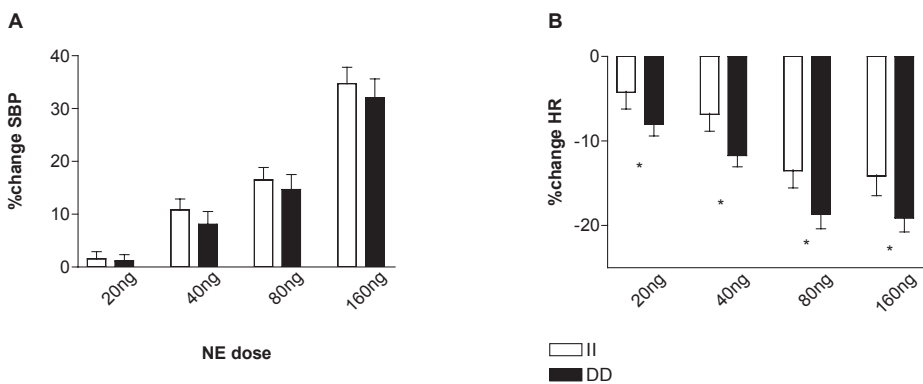


Table 2: Systolic bloodpressure (mmHg), mean arterial pressure (mmHg), heart rate (beats per min) and venous plasma norepinephrine (NE) levels (nmol/l) at baseline, during each NE infusion step and at recovery.

NE dose	Systolic blood pressure		Mean Arterial Pressure		Heart rate		Venous plasma NE	
	II	DD	II	DD	II	DD	II	DD
Baseline	119 ± 3	119 ± 3	85 ± 2	86 ± 2	65 ± 3	65 ± 2	1.1 ± 0.1	0.9 ± 0.1
20 ng	121 ± 2	120 ± 3	89 ± 2 ¹	89 ± 2 ¹	63 ± 3 ¹	60 ± 2 ^{1,3}	2.4 ± 0.2 ¹	2.2 ± 0.2 ²
40 ng	132 ± 2 ²	128 ± 4 ²	97 ± 2 ²	94 ± 3 ¹	61 ± 3 ¹	57 ± 2 ^{2,3}	3.5 ± 0.3 ²	3.1 ± 0.3 ²
80 ng	139 ± 2 ²	137 ± 5 ²	100 ± 2 ²	99 ± 3 ²	56 ± 2 ²	53 ± 3 ^{2,3}	6.5 ± 0.6 ²	6.2 ± 0.6 ²
160 ng	160 ± 3 ²	157 ± 5 ²	113 ± 2 ²	109 ± 4 ²	56 ± 2 ²	53 ± 2 ^{1,3}	13.7 ± 1.5 ²	12.0 ± 1.4 ²
Recovery	120 ± 3	124 ± 4	81 ± 2	85 ± 4	75 ± 3	71 ± 3	1.7 ± 0.2	1.2 ± 0.1

Data in mean ± SEM, ¹p<0.05 vs baseline, ²p<0.001 from baseline, ³p<0.05 overall from II

Table 3: Heart rate variability at baseline, during each norepinephrine infusion step and at recovery.

	ln(TP-HRV)		ln(LF-HRV)		ln(HF-HRV)		LF/HF ratio	
	II	DD	II	DD	II	DD	II	DD
baseline	8.1 ± 0.2	8.1 ± 0.2	6.9 ± 1.0	7.0 ± 0.7	7.0 ± 0.3	6.9 ± 0.2	1.1 ± 0.2	1.4 ± 0.4
20ng	8.3 ± 0.3 ¹	8.5 ± 0.1 ¹	6.9 ± 1.0	7.2 ± 0.5	7.3 ± 0.3 ²	7.4 ± 0.2 ²	0.8 ± 0.1 ¹	1.0 ± 0.2 ¹
40ng	8.6 ± 0.3 ²	8.6 ± 0.1 ¹	7.1 ± 1.1	7.3 ± 0.4	7.6 ± 0.3 ²	7.6 ± 0.2 ²	0.7 ± 0.1 ¹	0.9 ± 0.2 ¹
80ng	9.0 ± 0.3 ²	8.8 ± 0.3 ²	7.5 ± 1.3 ¹	7.3 ± 1.1	8.1 ± 0.3 ²	7.8 ± 0.3 ²	0.6 ± 0.1 ¹	0.7 ± 0.1 ¹
160ng	8.4 ± 0.3 ¹	9.0 ± 0.4 ²	6.8 ± 1.3	7.5 ± 1.3	7.5 ± 0.4 ²	8.1 ± 0.5 ¹	0.6 ± 0.1 ¹	0.7 ± 0.1 ¹
Recovery	7.8 ± 0.3	7.8 ± 0.3	6.5 ± 1.0	6.6 ± 1.0	6.5 ± 0.3	6.5 ± 0.3	1.1 ± 0.1	1.5 ± 0.4

Data in mean ± SEM, TP-HRV: total power, LF-HRV: low frequency power and HF-HRV: high frequency power of heart rate variability, see text for further explanation. Data: mean ± SEM, ¹= p<0.05 and ²= p<0.01 from baseline

Baroreflex sensitivity and heart rate variability

The spectral analysis-based BRS measurements at baseline did not differ between the groups (table 1, figure 3). The BRS responses during the NE infusions are shown in figure 3. BRS increased from baseline level at the first 3 infusion steps of 20, 40 80 ng/kg/min ($p < 0.001$ and $p < 0.05$, fig 3). At the highest NE infusion rate of 160 ng/kg/min, BRS reached a plateau in the *DD* group while it returned to baseline levels in the *II* group (fig.3). The gain in the spectral analysis based BRS was higher in the *DD* group at an NE infusion rate of 20 and 80 ng/kg/min ($p < 0.05$ vs *II* group). The overall difference in BRS between the groups, however, was close to significance (*DD* group vs *II* group, $P = 0.07$, fig 3).

The parameters of heart rate variability (HRV) during the NE infusion steps are shown in table 3. The total power of HRV significantly increased at each NE infusion step (table 3). This increase was primarily due to an increase in the HF-power of HRV in both groups (ANOVA, $p < 0.05$, table 2), while LF-power of HRV did not change significantly during the NE infusions (table 2). Consequently, the LF/HF ratio decreased with increasing NE-doses in both groups (ANOVA, $p < 0.01$, table 3). There were no between-group differences in the changes of either of the HRV parameters.

Discussion

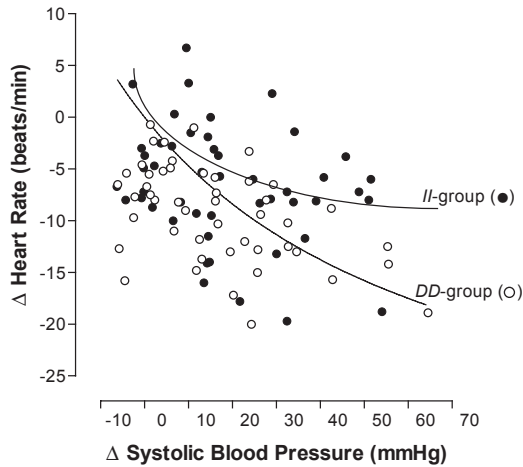
The primary objective of the study was to investigate whether intravenously administered pressor doses of NE induced different cardiovascular reflex responses in healthy persons with contrasting ACE-genotypes. Indeed, the NE-infusions induced a larger decline in heart rate in the subjects carrying the *DD* genotype with virtually no differences in blood pressure elevations between the groups. This strongly suggests that the baroreflex-mediated decline in heart rate was larger in *DD* subjects, which is supported by the steeper relationship between the increase in systolic blood pressure and the decline in heart rate in the *DD* group. The spectral-analysis based BRS was not different at baseline, but a large augmentation in BRS occurred during the NE-infusions. Interestingly, the gain in BRS in the *DD* group exceeded the *II* group at the 20 and 80 ng/kg/min NE-infusion step and remained elevated in the *DD* group at the highest NE-infusions rate of 160 ng/kg/min, while it declined to baseline levels in the *II* group. These findings suggest that during an acute challenge by exogenous NE, BRS may have more potential to increase in persons carrying the *DD* genotype. The changes in heart rate variability revealed no apparent

differences between the genotypes during the NE-infusions. Although we studied a relative small group of subjects, the current findings support the hypothesis that the ACE genotype influences the cardiovascular reflex responses to infusion of NE.

The aim of the baroreflex is short-term blood pressure control by modulation of parasympathetic and sympathetic influences on heart rate, cardiovascular contractility and peripheral vascular resistance. The system responds to small rises in blood pressure with an increase in vagal activity and a decrease in sympathetic tone, resulting in a deceleration of heart rate, a diminished contractility and a reduction of peripheral resistance. In this regard, the changes in BRS and HRV during NE infusion may have directly resulted from the NE-induced rises in blood pressure. The potentiation of the HF-frequency power, which was responsible for the increase in HRV, is in agreement with a baroreflex-mediated increase in parasympathetic nerve activity (18). The consequently reduced LF/HF ratio conjuncts with a shift in sympathetic/ parasympathetic balance towards a decreased sympathetic tone and increased vagal nerve activity (19). Thus, the observed changes are physiologically expected modulations in BRS and HRV in response to an exogenously induced rise in blood pressure (21), which should not be confused with the effects of endogenous sympathetic stimulation. As we have previously shown that standing, which causes endogenous sympathetic stimulation, provokes the opposite changes namely a drop in BRS and an augmentation of the HRV-LF frequency power (17). Our present study confirms and extends to a previous study, showing that an NE-infusion of 30 ng/kg/min for 6 hours caused a change in spectral analysis parameters compatible with a reduction in sympathetic activity (22). Thus, NE-infusions are most likely to decrease sympathetic nervous system activity of which its pre-existing firing rate may to some pact influence the degree to which BRS is reflectory augmented.

The non-invasive technique of spectral analysis allows continuous measurements of BRS under various circumstances (17), such as the incremental NE-pressure infusions applied in the present study. With this method, we demonstrated a large gain in BRS during the NE-pressor infusions. An increase in spectral analysis based BRS has also been reported in a study that infused NE with 100 ng/kg/min in healthy subjects (21). In our study, the highest NE-infusions dose of 160 ng/kg/min, induced no further rise in BRS in both ACE-genotype groups. Interestingly, BRS remained elevated in the *DD* group and declined to baseline in the *II* group. The duration of the NE-infusions of our study protocol could have been of relevance in the lack of further gain during increasing doses of NE. Baroreflex function serves short-term control of blood pressure, and starts to decline after 2 to 6 hours (23). Thus at the time we applied the 160 ng/kg/min NE-pressor infusion,

Figure 2. relation between norepinephrine induced change in systolic blood pressure and change in heart rate. Sign test on rank differences (10), $P < 0.05$ vs DD group.

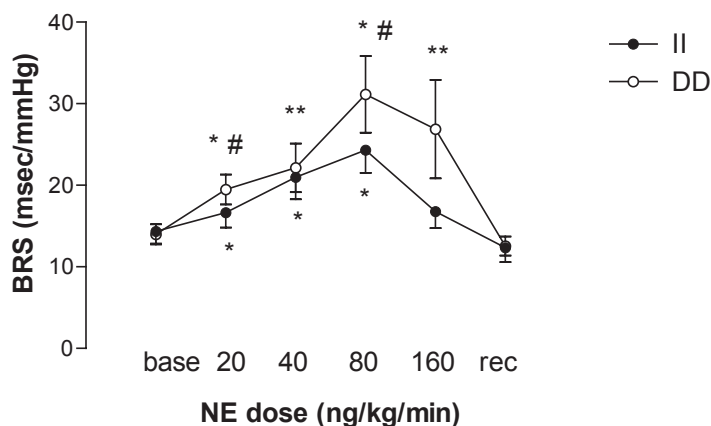


a further BRS stimulation may have been absent because of a time limited maximal stimulation of the baroreflex arc. The differences between the DD group and II group at the time of the assumed maximum stimulation of BRS is intriguing, as it raises the possibility that the DD genotype is associated with a larger functional reserve in BRS.

The main question that arises from our results is what mechanism is responsible for the more pronounced gain in BRS in the DD genotype with no differences in baseline BRS. One possibility is direct involvement of AngII. The DD genotype has an increased blood pressure response to AngI, suggesting that they have an increased propensity to the formation of AngII (3;4). AngII has powerful autonomic effects in animals as well as in man by modulating the function of the ANS at central and peripheral sites (24). Whether this could be involved in the more pronounced gain in BRS however, is doubtful, as blocking endogenous AngII in man, either by means of ACE inhibition or AT₁ receptor antagonism, improves baroreceptor function in normotensive subjects (25). It thus seems unlikely that the observed differences in the potentiation of BRS result from direct AngII related effects on BRS.

Indirect effects of the DD genotype should also be considered, as BRS reactivity may have been indirectly affected by an altered vasoconstrictor state in the DD subjects. Physiological AngII concentrations are not assumed to directly exert important tonic vasoconstrictor effects in healthy subjects, but both *in-vivo* and *in-vitro* physiologic studies

Figure 3. Baroflex sensitivity (BRS) response to infusion of increasing doses of norepinephrine (NE). Data: mean \pm SEM. * $p < 0.01$ and ** $p < 0.05$ from baseline, # $p < 0.05$ from II group.



have shown that AngII concentrations potentate adrenergic vasoconstrictor responses (26-28). Furthermore, AngII facilitates NE release from sympathetic nerve endings (24). On theoretical grounds, against a background of slightly increased AngII levels, one might expect an increased pressor response to a sympathetic stimulus like NE. In accord with this assumption, in patients undergoing extra-corporeal circulation during cardiac bypass surgery, an increased reactivity to phenylephrine *in vivo* was observed in DD subjects. Moreover, increased potentiating effects of AngII on vasopressor responses to phenylephrine in isolated artery segments of DD subjects *in vitro* were demonstrated (10). Other alterations in vascular function that have been linked to the DD genotype include a reduction of endothelium-dependent relaxation (29), an increased bradykinin degradation (30;31) and a different contribution of the NO system in vascular tone (9). Thus, several findings suggest that the determinants of vascular tone, as well as sensitivity to vasoconstrictor substances, may be altered in subjects carrying the DD genotype. These alterations may have played a role in the presently observed BRS augmentation provoked by NE. Our results are most likely explained by an increased vascular sensitivity to exogenous NE in DD subjects (9;10), that was adequately buffered by a larger BRS augmentation, and prevented a larger rise in blood pressure. The current study points toward an role of arterial baroreceptor function, that under circumstances of NE-induced vasoconstriction antagonizes neurohumoral vasoconstrictor forces the in DD genotype, possibly related to an impaired endothelial function in the DD genotype.

Perspectives

Exogenously administered NE-pressor infusions induced a larger baroreceptor mediated fall in heart rate in subjects homozygous for the *D*-allele of the ACE-gene polymorphism. This was further substantiated by spectral analysis based BRS measurements. These findings suggest that subjects carrying the *DD* genotype have a more effective potentiation of their BRS during a intravenous NE-challenge that could protect them against Ang II mediated increases in adrenergic vascular reactivity. Whether such a potential protects *DD* genotype positive persons against the development of hypertension, remains to be elucidated.

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